A Transport & Lairage model for *Salmonella* transmission between pigs, applicable to EU Member States.

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ABSTRACT

A model for the transmission of *Salmonella* between finisher pigs during transport to the abattoir and subsequent lairage has been developed, including novel factors such as environmental contamination and the effect of stress and is designed to be adaptable for any EU Member State (MS). The model forms part of a generic farm-to-consumption model for *Salmonella* in pigs, designed to model potentially important risk factors and assess the effectiveness of interventions. In this paper we discuss the parameterisation of the model for two case-study MSs. For both MSs, the model predicted an increase in the average MS level prevalence of *Salmonella* positive pigs during both transport and lairage, accounting for a large amount of the variation between reported on farm prevalence and reported lymph-node prevalence at the slaughterhouse. Sensitivity analysis suggested that stress is the most important factor during transport, while a number of factors including environmental contamination and the dose-response parameters are important during lairage. There was wide variation in the model predicted change in prevalence in individual batches; while the majority of batches (80-90%) had no increase, in some batches the increase in prevalence was over 70% and in some cases infection was introduced into previously uninfected batches of pigs. Thus, the model suggests that while the transport and lairage stages of the farm-to-consumption exposure pathway are unlikely to be responsible for a large increase in average prevalence at the MS level, they can have a large effect on prevalence at an individual batch level.

Keywords: Salmonella, Risk Assessment, Pigs, Transport, Lairage

1. INTRODUCTION

Salmonella infection is the second most common cause of foodborne illness in the European Union (EU) [1] and has been attributed to many sources, one of the main sources being pigs, [2, 3]. It is well known that many strains of *Salmonella* are endemic in the EU pig population [4], including several of the most common human serotypes. However, there is no confirmed relationship between infection in pigs and human illness, as many processes occur between the farm and human consumption that could affect the relationship. It is therefore of interest to develop a greater understanding of these processes in order to investigate where best to focus efforts to reduce *Salmonella;* whether at the pig farm, slaughterhouse, or in the home. To this end, in response to a European Commission mandate, a quantitative farm-to-consumption risk assessment for *Salmonella* in pigs was requested by the European Food Safety Authority (EFSA) [25]. This model provides an estimate for the risk of human illness from consumption of pork cuts, minced meat and fermented sausages. In this paper we discuss the Transport and Lairage component of this model.

Transport of pigs to the slaughterhouse and the subsequent lairage of pigs at the abattoir are thought to be important stages for *Salmonella* transmission in the pig production chain. It has been reported that there are significant increases in the prevalence of pigs infected with *Salmonella* between the farm and the slaughterhouse [5-7]. One such study reports trials that showed up to 20% of non-*Salmonella* shedding pigs within a batch were shedding *Salmonella* by the end of transport and lairage, through a combination of re-excretion and new infection [6]. While pigs are only in transport and lairage for a short period of time, research has shown that pigs from low risk herds are at risk of *Salmonella* infection when held in contaminated pens [8] and *Salmonella* can be isolated from the faeces of pigs exposed to a contaminated environment for as little as 2 hours [7, 9]. One study reports that 2-6 hours of combined transport and lairage could double the number of animals excreting *Salmonella* [6].

It is believed that during transport stress may play an important role in *Salmonella* transmission, causing an increase in faecal shedding [10] and carrier animals to revert to excreting *Salmonella* in their faeces [11, 12]. One study, while small, showed that even though rectal swabs of pigs on the farm and swabs of the truck prior to the entry of the pigs were all negative, 6 pigs were found to be excreting *Salmonella* after a 3 ¾ hours journey and all ten swabs of the truck after the journey tested positive for the same strain [11] . This indicates that environmental contamination is also an important factor to consider. Many studies have shown *Salmonella* spp. to be present in trucks used to transport pigs [13, 14], even after routine cleaning has been carried out [15, 16]. There are also numerous studies that have isolated *Salmonella* spp. in the lairage [14, 16, 17], where multiple batches of pigs can occupy the same living space in a short period (i.e. one day), with little or no cleaning between batches. Some studies have isolated *Salmonella* serovars from pigs that were present in the transport and lairage environments [14, 18], suggesting that they should be considered as potentially important sources of infection.

Modelling of infectious diseases is well recognised within both the veterinary and public health sectors as a useful tool for investigating the dynamics of pathogens within a population [19, 20]. Quantitative Microbiological Risk Assessments (QMRAs) are a useful modelling tool to assess the risk of an unwanted outcome and have been used in the field of food safety for the last ten years, particularly by government organisations. Indeed, a number of QMRAs on the subject of *Salmonella* in pigs over part, or the whole of, the farm to consumption chain in pork, have previously been developed [21-24].

In previous pig *Salmonella* QMRAs there has been little development of the transport and lairage stages, mostly relying on simple equations to model a proportional change in infection levels between the farm and slaughterhouse. However, as already stated, it has been established that pigs can become infected with *Salmonella* very quickly and certainly in less

time than the duration of transport or lairage. Also of concern is the fact that the skin of the pig could become contaminated with *Salmonella* once loaded into transport or lairage pens. It is therefore likely that there are many components of transport and lairage where interventions could take place to reduce the prevalence of infected pigs or concentration of *Salmonella* on contaminated skins. A mathematical model can be a useful tool to evaluate the effectiveness of these intervention strategies

These factors are the main driving forces behind this paper, where we propose a more in-depth framework to model the transmission of *Salmonella* during the transport and lairage of pigs. Such a model provides insight into the dynamics of *Salmonella* infection in finisher pigs at this stage and furthermore allows for the detailed modelling of intervention strategies implemented during these stages, such as the effect of separation of pigs and more effective cleaning of trucks and lairage, as discussed in a companion paper [(39)].

2. MATERIALS AND METHODS

2.1. Model overview

The Transport & Lairage model framework was designed to be applicable across the EU, with MS specific parameter estimates (e.g. the proportion of farms that are large, number of pigs slaughtered per day in a slaughterhouse) being used to parameterise for each specific MS. In this paper we present the results from two case studies (denoted MS1 and MS2), in order to demonstrate the parameterisation of the model for a high prevalence MS (i.e. slaughter pig *Salmonella* lymph node prevalence >20%) and a low prevalence MS (i.e. slaughter pig *Salmonella* lymph node prevalence < 5%). On the request of the EU, the MSs have been anonymised.

The model is stochastic in nature and simulates the transmission of *Salmonella* infection within batches of pigs during transport to the slaughterhouse and subsequent lairage. We define a batch to be a group of pigs that occupy the same 'living environment'; during transport this is a truck and during lairage a pen. In order to model cross contamination, the environmental contamination of the truck and the lairage environment is also simulated.

2.2. Model implementation

Each iteration of the model represents one day's worth of pigs going to one slaughterhouse. The model was implemented in Matlab R2010a (The MathWorks, 2010) and was run for 5,000 iterations, in order to capture the natural variation between both days and slaughterhouses (analysis on the convergence of mean values suggested that 5,000 was sufficient to capture all variation and achieve convergence). While variation is modelled, a decision was made not to include the uncertainty associated with model parameters, the effects of uncertainty are captured in a standalone analysis [26].

2.3. Model Framework

2.3.1. Initial conditions

To model the effect of transport and lairage we first need an estimate of the infection status of the finisher pigs in slaughter batches, as they leave a farm (i.e. are they susceptible or infected?). This estimate comes from the output of the farm model [27]. We assume (for simplicity and lack of data to the contrary) that pigs from large farms will go to large slaughterhouses and pigs from small farms will go to small slaughterhouses (where a large slaughterhouse is defined as one that slaughters more than 100,000 pigs per year). In this paper we only discuss the model in terms of pigs from the large farm going to a large slaughterhouse, details of how the model works for the small farms and slaughterhouses can be found in the EFSA report [25]. Thus, the input to the Transport & Lairage model is a database representing the *Salmonella* status of finishing pigs from 1000 farms, where from each farm 67 batches of pigs are sent to slaughter over the course of 500 days (determined

to be sufficient to capture the variability within the farm model and achieve convergence of results). The farms encompass a variety of different farm types (covering the majority of different farm types observed in the MS). For every batch of pigs sent to slaughter, the database stores information from the farm model on the infection status of every pig at the point of leaving the farm, (results are based on data on lymph-node infection), the concentration of *Salmonella* being shed in the faeces of each infected pig and the number of pigs in the batch.

2.3.2. Overview of the framework

The computational steps included in the model are shown in Figure 1 and an example of the movement of pigs between farm and slaughter is shown in Figure 2.

{Figure 1 and Figure 2 here}

At each iteration, the model assigns a specified number (or 'capacity') of pigs to be slaughtered, n_l, representing one day's worth of pigs to be slaughtered in a large slaughterhouse (this will vary between MSs and abattoirs within MSs, generally between 4000 – 15000 pigs). The model then randomly selects batches of slaughter-age pigs from the farm database (to capture the variation in prevalence between batches of pigs, both between farms and at different time points during the course of infection on a farm), until the total number of pigs selected is greater than or equal to n_l. The *Salmonella* status of the pigs in the selected batches is then entered into the Transport & Lairage model, where the transmission of *Salmonella* within these batches is modelled on an individual pig basis.

Following batch selection, the pigs are loaded onto the transport trucks. Data and expert opinion collected from MSs suggest that it is rare for a truck to pick up pigs from multiple farms in one journey the main exception being if two farms are owned by the same producer [(28)]. Thus, for simplicity, we make the assumption in the model that each truck will pick up a

165 week's worth of pigs from one farm only (one farm produces four batches of 40 slaughter-
166 age pigs, i.e. 160 pigs, per week).
167
168 Next we determine the duration of transport, $T_D(j)$, and the number of pigs in each 'pen', j, in
169 truck, i, $N_T(j,i)$, with a maximum cap on pigs in a pen, $\tau_{cap}(j)$. The pigs are loaded onto the
170 truck in batch order. The pigs within a batch are loaded into the first available pen in random
171 order. When a pen becomes full, the next pen is used. While there are several setups of
172 trucks that could be used (penned, non-penned, multi-layered), we assume that transport
173 time is sufficiently short so that there will not be sufficient opportunity for between-pen cross-
174 contamination. The differences between transport types are therefore negligible and each
175 pen with $N_T(j,i)$ pigs can be treated as a closed population. General practice is for all pigs that
176 are to be transported from a farm to be mixed together prior to loading, suggesting that any
177 division of pigs on the farm would not necessarily carry through to transport. Therefore, in the
178 model, the pigs are randomly mixed before being loaded onto the trucks.
179
180 The Lairage model simulates the transmission of *Salmonella* over the course of one day.
181 Pigs arrive at lairage and are unloaded into the lairage pens, with a maximum number of pigs
182 in a pen, L_{pencap}. The size of the lairage pens, L_{size}, which is important with regards to
183 environmental contamination, is estimated based on L_{pencap} and the stocking density of pigs
184 L_{stock}; $L_{size} = L_{pencap}/L_{stock,}$. We assume that the trucks arrive at the slaughterhouse over the
185 course of the day, during which time pigs that have arrived earlier will vacate the lairage pens
186 to enter the processing stages (pigs stay in the lairage pens for a duration of time, L_{time},
187 before moving into the slaughter process). Pigs that arrive later in the day will enter the pens
188 vacated by pigs that have already gone to be slaughtered. We assume that during this short
189 turnover the empty pen may undergo some cleaning (simple hosing down with water), but
190 more thorough cleaning (such as use of disinfectant) will only be done at the end of the day.
191 Pigs that arrive very late in the day may be held overnight, and slaughtered early the next
192 day. To model this we assume that L_O lairage pens will house pigs overnight and are

populated by as many batches of pigs as are needed to fill them. In the model, pigs housed overnight have a longer duration of stay (in a possibly contaminated lairage pen) and pens that house pigs overnight are not cleaned at the end of the day, affecting the probability that the pen is contaminated for subsequent batches of pigs.

2.3.3. Transmission of infection

During transport and lairage, we assume that a pig can be in one of two states at any time: susceptible (0) or infected (1). Thus, the infection status of pig *k*, in pen *j* at time *t* during stage *H* (where *H={T,L}* to denote transport and lairage respectively) is denoted by $\Omega_H(k,j,t)$, where $\Omega_H(k,j,t) \in \{0,1\}$. The average lymph node positive batch prevalence of pig infection is simply the mean of Ω_H. We define the variables $S_H(j,t)$ and $I_H(j,t)$ to be the total number of susceptible and infected pigs respectively, in pen *j* during stage *H,* at time *t*. We define the infected state to mean that a pig is infected in the ileo-caecal lymph-node and will intermittently excrete *Salmonella* in the faeces at varying concentrations, $c_p(j,k,t)$, ranging from 0 to 6 $\log_{10}$ cfu/g, in accordance with a previous study [29] and as modelled in the farm module [27]. During transport and lairage there are events that can cause either a change of state (e.g. susceptible pigs becoming infected) or a change in the concentration of *Salmonella* excreted by infected pigs.

To determine if a susceptible pig, *k,* in pen, *j,* at time *t* becomes infected, $\Psi_H(j,t,k)$, we use the beta-binomial dose-response model, as used for finishing pigs in the farm model [27]

$$\Psi_H(j,t,k) = \Re\left(B\left(1, p_{\inf}(j,t,k)\right), j,t,k\right),$$

where $\Re$ denotes a random sample taken from the distribution[1] and the probability of infection, p_{inf}, follows the beta-binomial dose response model

$$p_{\inf}(j,t,k) = 1 - \left(\left(1 - Beta\left(\alpha_{DR}, \beta_{DR}\right)\right)^{\lambda_H(j,t,k)}\right), \qquad (1)$$

[1] We use the terminology $\Re(X, j)$ to denote that a random sample is taken from distribution *X*, for every *j*. For example if *X* represents the binomial distribution and *j* represents pens, then a different number is randomly sampled from the binomial distribution for every pen.

with α_{DR} and β_{DR} the shape and scale parameters and $\lambda_H(j,t,k)$ the amount of *Salmonella* ingested by pig *k,* in pen *j*, at time *t*. If $\Psi_H(j,t,k)$=1 then the susceptible pig becomes infected.

We calculate $\lambda_H(j,t,k)$, by multiplying the amount of faeces (in grams) ingested by pig *k,* $m_{ing}(j,t,k)$ by the concentration of *Salmonella* in the ingested faeces, $c_H(j,t)$

$$\lambda_H(j,t,k) = \Re\left(Poisson\left(c_H(j,t)\right)\right) * m_{ing,H}(j,t,k), \quad (2)$$

where $m_{ing}(j,t,k) = \Re\left(Uniform\left(0, F_{eatMax}\right)\right)$, with F_{eatMax} the maximum amount of faeces ingested by a pig. We estimate $c_H(j,t)$ by dividing the amount of *Salmonella* in the environment, $E_H(j,t)$ by the amount of faeces in the environment, $F_H(j,t)$

$$c_H(j,t) = \frac{E_H(j,t)}{F_H(j,t)}. \quad (3)$$

Note that we assume that *Salmonella* and faeces will be homogenously spread throughout the pen.

When pigs enter the transport or lairage pens there is the possibility that these pens may be contaminated with *Salmonella* and/or faeces (we also consider the possibility of residual *Salmonella* on the floor/walls when there is no visible faecal material present, as one study found *Salmonella* in trucks that were not considered visibly contaminated with faeces [15]). We define this contamination as 'carryover'. Thus, to estimate $F_H(j,t)$, we sum the environmental carryover of faeces, $F_{carry,H}(j,t)$ (described in more detail in Section 2.3.4), and the total faeces excreted by pigs in pen *j*, $F_{pig,H}(j,t)$ (described in more detail in Section 2.3.5)

$$F_H(j,t) = F_{carry,H}(j,t) + F_{pig,H}(j,t). \quad (4)$$

Similarly, $E_H(j,t)$, is estimated by summing the number of *Salmonella* in the environmental carryover $E_{carry,H}(j,t)$ (described in more detail in Section 2.3.4) and the total *Salmonella* excreted by infected pigs, $E_{pig,H}(j,t)$

$$E_H(j,t) = E_{carry,H}(j,t) + E_{pig,H}(j,t). \quad (5)$$

A schematic of the transmission dynamics during transport and lairage is shown in Figure 3, using the notations already defined in this section.

{Figure 3 here}

2.3.4. Initial pen conditions – carryover

For each truck and lairage pen, the model determines whether or not contamination has been carried over from the previous batch of pigs. If it has been carried over then the quantity is determined. We define $F_{carry,H}(j,t)$ as the amount of faeces (g) left in pen *j* at time *t*, and $E_{carry,H}(j,t)$ as the amount of *Salmonella* (cfu) left in pen *j*, at time *t*, where *t* is a discrete time interval corresponding to the time at which the t^{th} batch of pigs occupy the pen on a given day (note that for transport, *t*=1 at all times, as we do not consider multiple occupations of transport pens in a given day).

For transport, it was not possible, due to lack of data, to directly consider the prior history of the truck (e.g. what animals were in the truck before? How many animals were in the truck? Were they infected with *Salmonella*? Was the environment contaminated?). We estimate $F_{carry,T}(j,t)$ and $E_{carry,T}(j,t)$ from studies that record the frequency and degree of contamination of trucks before the pigs are loaded. Assuming independence between trucks

$$F_{carry,T}(j,t) = \Re\left(B(1,1-p_{FaecCarry,T}), j, t\right) * \Re\left(U(1, F_{TransMax}), j, t\right), \quad (6)$$

where $p_{FaecCarry,T}$ is the probability that the truck has been successfully cleaned and all faecal contamination has been removed and $F_{TransMax}$ is the maximum amount of faeces carried over. Similarly

$$E_{carry,T}(j,t) = \Re\left(B(1,1-p_{EnvCarry,T}),j,t\right) * \Re\left(U(1,E_{TransMax}),j,t\right), \quad (7)$$

where $p_{EnvCarry,T}$ is the probability that the truck has been successfully cleaned and all *Salmonella* removed and $E_{TransMax}$ is the maximum amount of *Salmonella* present in the truck when pigs enter.

We estimate the capacity of lairage as a proportion of the throughput of pigs for the day and then simulate the lairage over the course of the day, thus allowing for events such as cleaning between batches to occur. Thus the model provides an estimate of the prior history of the pens when new pigs are placed in them. However, we do not know the history of the pen for the first batch of the day, so for *t*=1 we use a similar method as during transport to estimate the amount of *Salmonella*, $E_{carry,L}(j,t)$, and faeces, $F_{carry,L}(j,t)$, in pen *j,* at time *t*, from studies that record the frequency and degree of contamination of lairage pens. Therefore, assuming independence between pens

$$F_{carry,L}(j,t) = \begin{cases} \Re\left(B(1,1-p_{FaecCarry,L}),j,t\right) * \Re\left(U(1,F_{LairMax}),j,t\right) & t=1 \\ F_L^c(j,t-1) - F_L^c(j,t-1) * \Re\left(B(1,p_{clean,L}),j,t\right) * \chi_L^F(j,t) & t>1 \end{cases}, \quad (8)$$

where $F_L^c(j,t-1)$ is the amount of faeces left in the pen after previous occupation, $\chi_L^F(j,t)$ is the proportion reduction of faeces due to cleaning and $p_{clean,L}$ is the probability that the pen is cleaned. The amount of *Salmonella* in a lairage pen is estimated by

$$E_{carry,L}(j,t) = \begin{cases} \Re\left(B(1,1-p_{EnvCarry,L}),j,t\right) * \Re\left(U(1,E_{LairMax}),j,t\right) & t=1 \\ E_L^c(j,t-1) - E_L^c(j-1) * \Re\left(B(1,p_{clean,L}),j,t\right) * \chi_L^E(j,t) & t>1 \end{cases}, \quad (9)$$

where $E_L^c(j,t-1)$ is the load of *Salmonella* left in the pen after previous occupation and $\chi_L^E(j,t)$ is the proportion reduction of *Salmonella* due to cleaning.

2.3.5. *Amount of faeces in a pen*

The amount of new faeces excreted in pen *j* at time *t*, $F_{pig,H}(j,t)$, is estimated by summing up the amount of faeces excreted by all pigs currently in pen *j*

$$F_{pig,H}(j,t) = \sum_{k=1}^{N_H(j,t)} f_{pig,H}(k,j,t), \quad (10)$$

where $N_H(j,t)$ is the total number of pigs currently in pen *j*. The amount of faeces excreted by pig *k* in pen *j* at time *t,* is estimated as

$$f_{pig,H}(k,j,t) = \Re\left(\bar{f}(k,j,t)\right) * \Re\left(B\left(T_H^D(j,t), P^D\right), j\right), \quad (11)$$

where $\bar{f}(k,j,t)$ is the amount of faeces excreted by pig *k* in pen *j* per defecation, P^D is the probability of a defecation per hour and $T_H^D(j,t)$ is the duration of time (integer number of hours) the batch of pigs spend in pen *j* at stage *H.*

2.3.6. *Amount of Salmonella in a pen*

The *Salmonella* excreted by infected pigs in pen *j* at time *t*, $E_{pig,H}(j,t)$, is given by the formula

$$E_{pig,H}(j,t) = \sum_{k=1}^{N_H(j,t)} f_{pig,H}(k,j,t) * c_p(k,j,t), \quad (12)$$

where $c_p(k,j,t)$ is the concentration of *Salmonella* (cfu/g) excreted in the faeces by pig *k,* which is an output from the farm module [27].

2.3.7. *Effect of Cleaning and Disinfection*

During transport and lairage pigs are kept in confined spaces and in close contact. One study [30] reported a mean stocking density of pigs of 239 kg/m^2 for full truck loads in winter (standard deviation of 38). This high stocking density means that there is a high risk of exposure to *Salmonella* contaminated faeces. This risk is further heightened by the likelihood of carryover from previous batches of pigs, as while trucks may be cleaned between journeys it is reported that this cleaning will not remove all of the *Salmonella* from a contaminated

vehicle [13, 15]. However, different methods of cleaning have different effects [31]. We also take account of the fact that the type of cleaning employed at the end of the day is often more rigorous and so the proportion reduction of *Salmonella* in the pen due to cleaning at this time is considered to be more effective [32]. If pigs are housed overnight in a pen, then the estimates for within-day cleaning are used to provide an estimate of carryover for the next batch of pigs.

2.3.8. Effect of stress during transport

To account for the effect of stress we assume that there is a fixed probability, p_{rex}, that stress will affect the shedding of *Salmonella* in already infected pigs during transport. This probability includes the effect of stress caused prior to transport, when pigs may be held on the farm overnight in new housing or mixed with unfamiliar pigs. There is little evidence to suggest that stress is such an important factor during lairage and in fact longer lairage times have been reported to be beneficial in reducing the previous stress of transport [33]. Thus, we do not consider stress in lairage.

A US study looked at the effect of mixing (social) stress on populations of *Salmonella* Typhimurium in segregated early weaning pigs [34]. After 5 days they found that the incidence of faecal *Salmonella* shedding was higher in mixed contact pigs. They concluded that social stress of weaned pigs may increase susceptibility to and/or faecal shedding of *Salmonella*. This study is not directly related to transport stress, but it does suggest the effect that stress will have on pigs infected with *Salmonella.* Therefore, in the absence of other relevant data, we assume that the concentration of *Salmonella* excreted in the faeces of stressed pigs will be increased. To model this, we change the distribution for concentration of *Salmonella* excreted in the faeces of stressed pigs, so that higher concentrations are more likely and consequently, under stress, more infected pigs will be excreting *Salmonella*. There are little data to determine exactly how or when we should change this distribution. One study found there to be an observable difference in excretion levels between pigs infected with a low

dose of *Salmonella* and those infected with a high dose [29]. Given the lack of data, we assume that the effect of stress is equivalent to the difference between excretion levels of low dose and high dose pigs (estimated as between 1-3 log cfu/g). Thus if the model determines that stress is affecting a pig during transport at time *t*, the amount of *Salmonella* they shed, $\Phi_{stress}(j,k,t)$, is estimated by increasing the current amount shed by between 1-3 log cfu/g (determined by a random sample from a U(1,3) distribution), but with a maximum of 6 log cfu/g (so a pig that was already shedding would not increase to any more than 6 log cfu/g)); $\Phi_{stress}(j,k,t) = Max\left(c_p(j,k,t) + \Re(U(1,3)),6\right)$.

2.4. Sensitivity Analysis

To determine the extent to which the variability of the baseline model parameters affects the model output, we conducted a one-way analysis of variance (ANOVA). The ANOVA method is a standard statistical method that has previously been used as a method for sensitivity analysis of food safety risk assessments [35, 36] and the methodology is discussed in detail elsewhere [37]. Briefly, for each iteration *y* of the model the ANOVA analysis compares a point estimate of the input parameter value against the value of a 'response' variable, returning an F value which provides a measure of the extent to which the two are correlated (Note that many parameters take multiple values during an iteration, such as duration of transport which has a different value for each truck. Therefore, we take the point estimate to be the average of all the values of the input parameter during iteration *y*). We conduct two sensitivity analyses for each MS, one for transport and one for lairage. For the transport sensitivity analysis we use the average lymph-node positive prevalence per truck at the end of transport as the response variable and for the lairage sensitivity analysis we use the average lymph-node positive prevalence per batch (batch defined as a group of pigs that occupy a lairage pen at the same time) at the end of lairage as the response variable.

2.5. Parameter Estimation

Parameter estimates are shown in Table I-Table V. Further assumptions made for parameter estimates are given below (for full details of the parameter estimation see the full EFSA report [25]).

{Table I-Table V here}

2.5.1. Amount of faeces excreted, $\bar{f}(k, j)$

To calculate the amount of faeces shed we estimate the number of defecations while in the pen and the amount of faeces excreted in each defecation. Data from a study records the number of times pigs excrete per day by weight class [38]. As we are modelling finishing pigs we use the 105kg weight class (the largest weight), which were found to excrete on average 3.1 times per day. Data collected for the farm module suggests that the amount of faeces shed by a finisher pig per day has a mean of 2580g and a standard deviation of 50g [27]. We fit a gamma distribution to these values (as the amount of faeces shed per day cannot be negative). To determine the amount shed by a particular pig, *k,* in pen *j*, per excretion, $\bar{f}(k, j)$, we sample from this distribution for each individual pig and then divide the answer by 3.1 (the average number of times finisher pigs excrete per day), see Table I.

2.5.2. Probability of transport stress, p_{rex}

No data are available to estimate this parameter from published data. Expert opinion (AHVLA, 2008) suggests that on farm, pigs would revert to shedding from a carrier status (defined as infected but not excreting *Salmonella*) around 10% of the time. We assume the carrier status is analogous to the infected animals in the current model that are either not shedding *Salmonella* or shedding at a low-level (<2 log cfu/g) and that the increase in shedding observed during transport is simply these low-level shedders excreting enough to test positive again (appearing as carriers reverting to excretion). As stress during transport is assumed to increase this rate and in the absence of any other data, we double this estimate to p_{rex}= 20%.

To estimate the probability of an excretion per hour we divide 3.1 by the number of hours a day a pig is active (and thus able to excrete). We assume this to be 12 hours and so estimate the probability of an excretion per hour to be P^D = 3.1/12=0.2583.

2.5.3. Effectiveness of cleaning in lairage, χ^E_L

There are many different types of cleaning that could be implemented to clean out lairage pens (e.g. pressure washing, steam washing, use of sanitiser). Qualitative data from the UK suggests that most premises use pressure washing or steam-cleaning [(32)]. A laboratory study was conducted on the log reduction of *Escherichia coli* counts using different cleaning methods on either a visually clean or visually dirty concrete slab [(31)]. Log_{10} reductions were recorded immediately after cleaning and again one hour after. The mean reductions and standard deviations are reported. We fit normal distributions to these data (see Table III) assuming that the immediate reduction is applicable to cleaning out between batches of pigs during the day and the reduction after an hour is applicable to overnight cleaning. We assume that all premises will use either pressure washing or steam cleaning with equal probability and estimate the log reduction in contamination due to cleaning during the day. Note that this estimation assumes that the proportion reduction in *E. coli* counts is equivalent to the proportion reduction in *Salmonella* counts.

3. RESULTS

Table VI shows the average lymph node positive batch prevalence of pig infection for the two MSs, before transport, after transport and after lairage. It can be seen that MS2 has the highest prevalence at each stage, with an average prevalence of 20% at the end of lairage, while MS1 is only 1%. The average prevalence increases between transport and lairage for both MSs. The 5th and 95th percentiles of batch prevalence show that there is a large degree

411 of variation between days, with the average lymph node positive batch prevalence for some
412 days reaching almost 3% for MS1 and 35% for MS2.
413 {Table VI here}
414 Over 80% of batches showed no change in prevalence during transport and lairage. Figure 4
415 shows the distribution for the nonzero increases in lymph node positive batch prevalence
416 during transport and lairage for both MSs. Most batches show a small increase (<10%), but a
417 few batches show more than a 50% increase in lymph node positive prevalence. The
418 distributions suggest that there are more higher prevalence increases during lairage than
419 during transport. Additional analysis (not shown here) shows that when there is an increase
420 in prevalence, even when there are only few animals infected in a batch at the farm, there
421 can be over 50 extra animals infected after transport.
422 {Figure 4 here}
423 Figure 5 shows the results of the transport and lairage sensitivity analyses for MS1 and MS2
424 and full descriptions of the labels are in Table IV and Table V. We plot the F value, so the
425 bigger the bar the more significant the variation in the parameter is on the lymph-node
426 positive prevalence at the end of transport (although factors with bars of similar height should
427 be considered equally significant). For transport, it is clear that stress (*T4)* is the most
428 important factor in our model for both MSs. Stocking density (*T3*) is also relatively important
429 for MS1. Note that the initial batch prevalence is not included as a factor as it is an output of
430 the previous farm model. However if it is included it is by far the most important factor (with
431 an F value around 5 times that of stress, results not shown here). This suggests that the on
432 farm within batch prevalence is more influential on the mean lymph-node positive batch
433 prevalence at the end of transport than the factors which influence a change in prevalence
434 during transport (such as stress and environmental contamination). However, the model
435 does show that there can be a large change in individual batch prevalence due to transport
436 factors.
437 {Figure 5 here}

438 The results of the lairage sensitivity analysis showed that the significance of the parameters
439 differ between MSs. For MS1 it is whether pigs are kept overnight (*L1*) that is most important
440 while for MS2 it is whether *Salmonella* is carried over in the pens between batches (*L4*). It is
441 clear that many of the parameters have similar significance on the prevalence at the end of
442 lairage and it is not just one parameter that overwhelms everything else (as stress seems to
443 during transport). Again we do not include the batch prevalence at the beginning of lairage as
444 a parameter. When it is included it is much more significant than the other parameters (with
445 an F value around 15 times higher than keeping pigs overnight), as the farm prevalence is in
446 transport, again suggesting that the previous within batch prevalence is highly influential.
447

448 # 4. DISCUSSION

449 A stochastic model for the transmission of *Salmonella* between pigs in the Transport &
450 Lairage stages of the pig farm-to-consumption chain has been developed. The model
451 framework is adaptable to any EU Member State, with appropriate data, and is part of a
452 generic farm-to-consumption model. This model has been developed to incorporate factors
453 that are thought to influence the prevalence of *Salmonella* in slaughter-age pigs, including
454 stress during transport, contamination of the environment and cleaning of the environment.
455 These factors were included with the aim of assessing the effect of various interventions
456 implemented at the transport and lairage stages on the risk of human *Salmonella* infection.
457 This analysis is discussed elsewhere [(39)].
458

459 We can validate the results of the model by comparing the average lymph-node positive
460 prevalence at the end of lairage for each MS with the corresponding lymph-node positive
461 prevalence given in the EFSA slaughter pig baseline survey [(4)]. The model results matched
462 the EFSA survey to within a tolerance of 1%. For MS2 the EFSA baseline survey results
463 gave a mean prevalence of 21.2% (5^{th} and 95^{th} percentiles of 17.8% and 25% respectively),
464 while the QMRA predicted a mean prevalence of 20%, well within the 5^{th}-95^{th} percentile

range. For MS1, EFSA baseline results gave a mean prevalence of 2% (5^{th} and 95^{th} percentiles of 1.1% and 3.6% respectively) while the QMRA predicts a mean prevalence of 1%, just below the 5^{th} percentile. This suggests that, while the model captures the factors thought to be of most importance to *Salmonella* transmission and prevalence during transport and lairage, these factors alone do not completely explain all the variability in the system. While the model may be less accurate for low prevalence MSs, such a difference between the model results and observed results does not have a great impact on the predicted number of human cases of the full model.

As with most risk assessments, we encountered a number of data gaps during the parameterisation of the model. Perhaps the most important data gap was the effect of stress during transport. There is little quantitative data on stress so expert opinion had to be used to estimate the proportion of pigs that become stressed. On top of this, the effect that stress has in relation to *Salmonella* is not clear. We have assumed that it will result in a 1-3 log cfu/g increase in the amount of *Salmonella* shed in the faeces of lymph-node positive pigs; [29]. While no data are perfect, the lack of available data on the amount of *Salmonella* and faeces that would be carried over (i.e. amount present in the pen prior to entry of the pigs) was also of concern. There are reasonable data on whether *Salmonella* was isolated from a pen/truck before pigs enter it, but the data on how much is present is limited.

As well as being a significant data gap, the sensitivity analysis suggested that the number of stressed pigs in a batch during transport was significant. Furthermore, as the model assumes stressed pigs increase the amount of *Salmonella* shed in the faeces, this can have an effect during the slaughter process; higher loads of *Salmonella* would be released if a cross-contamination event occurs, resulting in higher concentrations of *Salmonella* on contaminated carcasses.

492 The amount of *Salmonella* and faeces that would be carried over and housing pigs overnight
493 were also considered significant factors in the sensitivity analysis. The longer time spent in
494 lairage when housed overnight leads to increased risk of *Salmonella* infection if the pigs are
495 in a contaminated environment or share accommodation with infected pigs. However, a
496 model simulation where environmental contamination was reduced by 2 logs (to simulate
497 more effective cleaning and thus reduce the impact of carryover and environmental infection)
498 did not have much of an effect at reducing human infection [(39)]. This indicates that the
499 prevalence of infected pigs in a batch is a more important factor than environmental
500 contamination. As such, the effect of the initial on-farm prevalence should not be overlooked.
501 This factor, when included in the sensitivity analysis, was by far the most significant,
502 suggesting that on-farm control measures that lead to lower within or between batch
503 prevalence at the start of transport could be more effective than control measures
504 implemented during transport or lairage. The full QMRA suggests that it is more effective to
505 control the prevalence of *Salmonella* infected pigs by interventions on the farm or the
506 prevalence of contaminated carcasses during the slaughter process [(25)]. However, this
507 analysis did suggest that changing the probability of stress has a noticeable effect on the
508 model predicted risk of human illness. For example the risk for pork cuts in MS2 was reduced
509 by 27% when stress was halved (i.e. p_{rex}=10%). This may be in part due to the concentration
510 of *Salmonella* on carcasses and in pig faeces being the main factors that affect human
511 illness (stress affects this, while the dose-response does not). This highlights how important
512 it is that accurate data for stress are obtained and utilised within the model.
513
514 One caveat to these conclusions is that, due to lack of data, it was not possible to look at
515 skin contamination during transport or lairage. We had initially hoped to model the change in
516 skin contamination during transport and lairage, but while there are many studies that report
517 the prevalence of carcass contamination during the slaughter process [(4)], very few actually
518 record the prevalence at the start of processing (i.e. immediately post-lairage) and no
519 information could be found on prevalence of skin contamination during transport or lairage. In

520 the full model, skin contamination is estimated at the start of the slaughterhouse process
521 using a simple equation relating lymph-node positive prevalence to skin contamination
522 prevalence. Due to this simplification, the Transport & Lairage model would miss any
523 potential effect of interventions that would affect skin contamination independently of lymph
524 node prevalence. For example, more effective cleaning of the lairage pens will have a
525 greater effect on contaminated skins, than lymph-node positive status. Until there are reliable
526 data to accurately estimate the level of skin contamination during the lairage process the
527 effect of interventions on the level of skin contamination cannot be modelled. Other possible
528 limitations of the model include not modelling cross-contamination between pens during
529 transport or lairage and the assumption of no mixing of pigs from different farms during
530 transport. In both cases the limited available data suggested that these events were unlikely
531 to happen. The limited exposure time was considered to make infection via cross-
532 contamination unlikely. If good data become available for these variables in the future, then it
533 would be interesting to simulate their impact on the *Salmonella* prevalence in the model. The
534 choice of using lymph node prevalence to explain *Salmonella* infection may also impact the
535 results, as other measures may give alternative initial prevalence estimates.
536
537 Previous research has suggested that control measures to decrease the *Salmonella* risk for
538 food safety would be best implemented on-farm at the finishing stage or during the slaughter
539 process [(23)]. Analysis of the full farm-to-consumption QMRA, of which the Transport and
540 Lairage model is a part, also predicted that control measures implemented on-farm and at
541 the slaughterhouse would have the greatest effect [(39)]. However, the results from this model
542 do suggest that both within and between batch prevalence of *Salmonella* infection can
543 increase, during both transport and lairage. Increases were observed in about 10% of
544 batches for MS1 and 20% of batches for MS2. While the majority showed a relatively small
545 increase (<10%), in a few cases this increase was much higher (>50%). Additionally, around
546 5% of batches of pigs became infected during transport (i.e. there were no infected pigs in
547 the batch upon leaving the farm, but at least one infected pig in the batch by the end of

548 transport). Therefore, while this model does not suggest that the Transport and Lairage
549 stages should be the main stages to focus on for decreasing the *Salmonella* risk for food
550 safety, it does suggest that they can be an important, albeit relatively infrequent, source of
551 infection for batches of pigs. Also, these stages may be more influential in MSs with relatively
552 high *Salmonella* slaughter-pig prevalence, so control measures during Transport and Lairage
553 may be less appropriate in relatively low prevalence MSs.
554

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560

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564

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713

Tables

Table I: Global parameter estimates/definitions: values are for both Member States (MS1 and MS2), unless specified.

Parameters	*Description*	*Value used in simulations*	*Reference*
$n_l(q)$	Number of pigs to be slaughtered at large abattoir	MS1: $\Re\left(Uniform\left(4000,5000\right)\right)$ MS2: $\Re\left(General\left([1{,}5000,\ 10000,15000],\ [16,\ 5,\ 1]/22\right)\right)$	(28)
$\overline{f}$	Average amount of faeces shed by pig per defecation	$\bar{f}(i,k)=\frac{\Re\left(Gamma\left(\frac{2580}{50^2},\frac{2580^2}{50^2}\right)\right)}{3.1}$	(40)
P^D	Mean number of defecations per hour	$\frac{3.1}{12}$ defecations / hour	(38)
$c_p(i,j,k)$	Concentration of *Salmonella* (cfu/g) shed by pig *i*	Initial estimates from farm model	(27)
$\alpha_{pigD}, \beta_{pigD}$	parameters for pig dose response model	$(\alpha_{pigD}, \beta_{pigD})$ = (0.1766, 20235)	(41)
F_{eatMax}	Maximum amount of faeces eaten by pig	$\frac{100}{12}$ g/hour	Assumed by author based on expert opinion

Table II: Transport parameter estimates/definitions: values are for both Member States (MS1 and MS2)unless specified.

Parameters	*Description*	*Value used in simulations*	*Reference*
p_{rex}	Probability of pig becoming stressed during transport	0.2	Assumed by author based on expert opinion
$\tau_{cap}(j)$	Number of pigs in pen in transport	MS1: $\Re(BetaPert(10,12.5,15))$ MS2: $\Re(Uniform(14,20))$	(28) (42)
$P_{EnvCarry,\ T}$	Probability of environmental carry over in truck	5/18	(15, 22)
$P_{FaecCarry,\ T}$	Probability of faeces carry over on truck	1/9	(15)
$F_{TransMax}$	Maximum faeces carry over in transport (g per truck).	990g	(43)
$E_{TransMax}$	Maximum *Salmonella* carried over in transport	$\Re(Uniform(0,0.11))$ cfu/cm^2	(15)

$\chi^{E}{}_{T}(k,j)$	Proportion reduction of *Salmonella* due to cleaning	0.621	[22]
$\chi^{F}{}_{T}(k,j)$	Proportion reduction of faeces due to cleaning	0.621	[22]
$T_D(j)$	Duration of transport (minutes)	MS1: $\Re(BetaPert(30,60,480))$ MS2: Empirical distribution fit to data; mean time 60.71 (95% CI [59.46, 61.95])	[28] AHVLA unpublished data from Animal movements licensing scheme

Table III: Lairage parameter estimates/definitions: values are for both Member States (MS1 and MS2) unless specified.

Parameters	*Description*	*Value used in simulations*	*Reference*
L_{pencap}	Number of pigs in a pen in lairage	50	[17]
L_{stock}	Stocking density of pigs (pigs/cm^2)	$\Re(Uniform(0.42/10000, 0.83/10000))$	[44]
$L_{time,Day}$	Time (hrs) spent in lairage during day	$\Re(Gamma(2.8,7.84))$	[32]
$L_{time,Night}$	Time (hrs) spent in lairage if kept overnight	$\Re(Gamma(3.83,58.52))$	[32]

$P_{overnight}$	Probability of number of pens used for overnight stay	Discrete distribution : [0pens, 1pen, 2pens]= [0.2 0.7 0.1]	(32)
$P^{L}_{envLair}$	Probability environmental carryover in lairage	51/150	(5, 17, 22)
$Max_{envLair}$	Max *Salmonella* carry over in lairage	550/100	(17)
P^{L}_{clean}	Probability pen is cleaned between batches	0.25	(32)
$\chi^{E_L}(j,t)$	Reduction in *Salmonella* due to cleaning during the day (Log10)	$\chi^{E_L}(j,t)=\begin{cases}\Re(N(2.5,0.7),j), & y<0.5\\ \Re(N(0.9,0.7),j), & y\geq 0.5\end{cases}$	(31)
$\chi^{E_L}(j,t)$	Reduction in *Salmonella* due to cleaning overnight (Log10)	$\chi^{E_L}(j,t)=\begin{cases}\Re(N(4.1,1.7),j), & y<0.5\\ \Re(N(1.7,1.6),j), & y\geq 0.5\end{cases}$,	(31)
$P_{FaecCarry,\,L}$	Probability carryover of faeces	8/10	(17)
$\chi^{F_L}(j,t)$	Reduction in faeces due to cleaning	0.019	(22)

Table IV: Transport sensitivity analysis parameters

Parameter	*Description*
T1(i)	Duration of transport for truck *i*
T2(i)	Pen capacity in truck *i*
T3(i)	Average stocking density in truck *i*
T4(i)	Average prevalence of stressed pigs per pen in truck *i*
T5(i)	Number of pens with *Salmonella* carryover in truck *i*
T6(i)	Average amount of *Salmonella* carried over per pen in truck *i*
T7(i)	Average amount of faeces shed by pigs in truck *i.*
T8(i)	Number of pens with faecal carryover in truck *i*
T9(i)	Average amount of faeces carried over per pen in truck *i*
T10(i)	Average concentration in faeces of pigs per pen in truck *i*
T11(i)	Average probability of illness for pigs in truck *i*

Table V: Lairage sensitivity analysis parameters

Parameter	*Description*
L1(l)	Is batch *l* in lairage overnight? - {yes, no}
L2(l)	Type of washing used in pen before batch *l* enters - {pressure washing, steam washing}
L3(l)	Amount of *Salmonella* in pen before batch *l* enters (carryover)
L4(l)	Is there any *Salmonella* carryover? – {yes, no}
L5(l)	Is there any faecal carryover? – {yes, no}
L6(l)	Amount of faeces in pen before batch *l* enters (carryover)
L7(l)	Amount of faeces shed by batch *l* during lairage.
L8(l)	Duration of time batch *l* spent in lairage
L9(l)	Size of lairage pen occupied by batch *l*

L10(l)	Reduction of *Salmonella* contamination of pen due to cleaning, before batch *l* enters
L11(l)	Average probability of illness for pigs in batch *l*

Table VI: Mean, 5th and 95th percentiles of model predicted lymph node positive batch prevalence before transport, after transport and after lairage for both Member States (MS1 and MS2).

	Mean, (5th, 95th percentiles) of prevalence (%)		
Member State	**Before transport**	**After transport**	**After lairage**
MS1	0.43 (0.08, 1.03)	0.62 (0.12, 1.38)	1 (0.2, 2.7)
MS2	16.5 (3.1, 29)	17.6 (4.1, 30.2)	20 (4.9, 35.4)

Figures

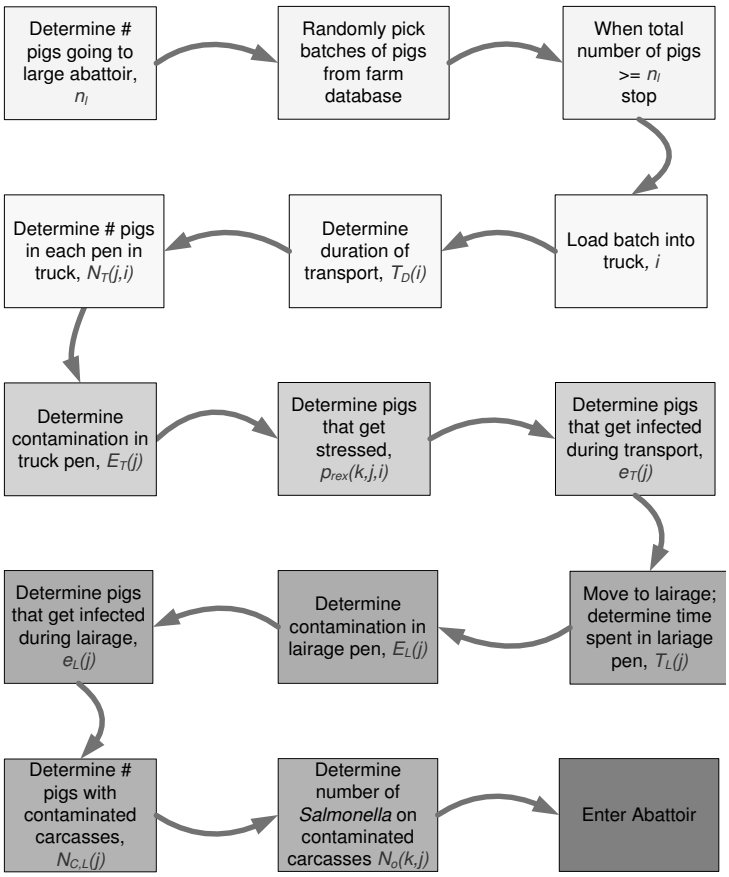

Figure 1: Computational steps in the Transport & Lairage simulation model (for pigs from a large farm).

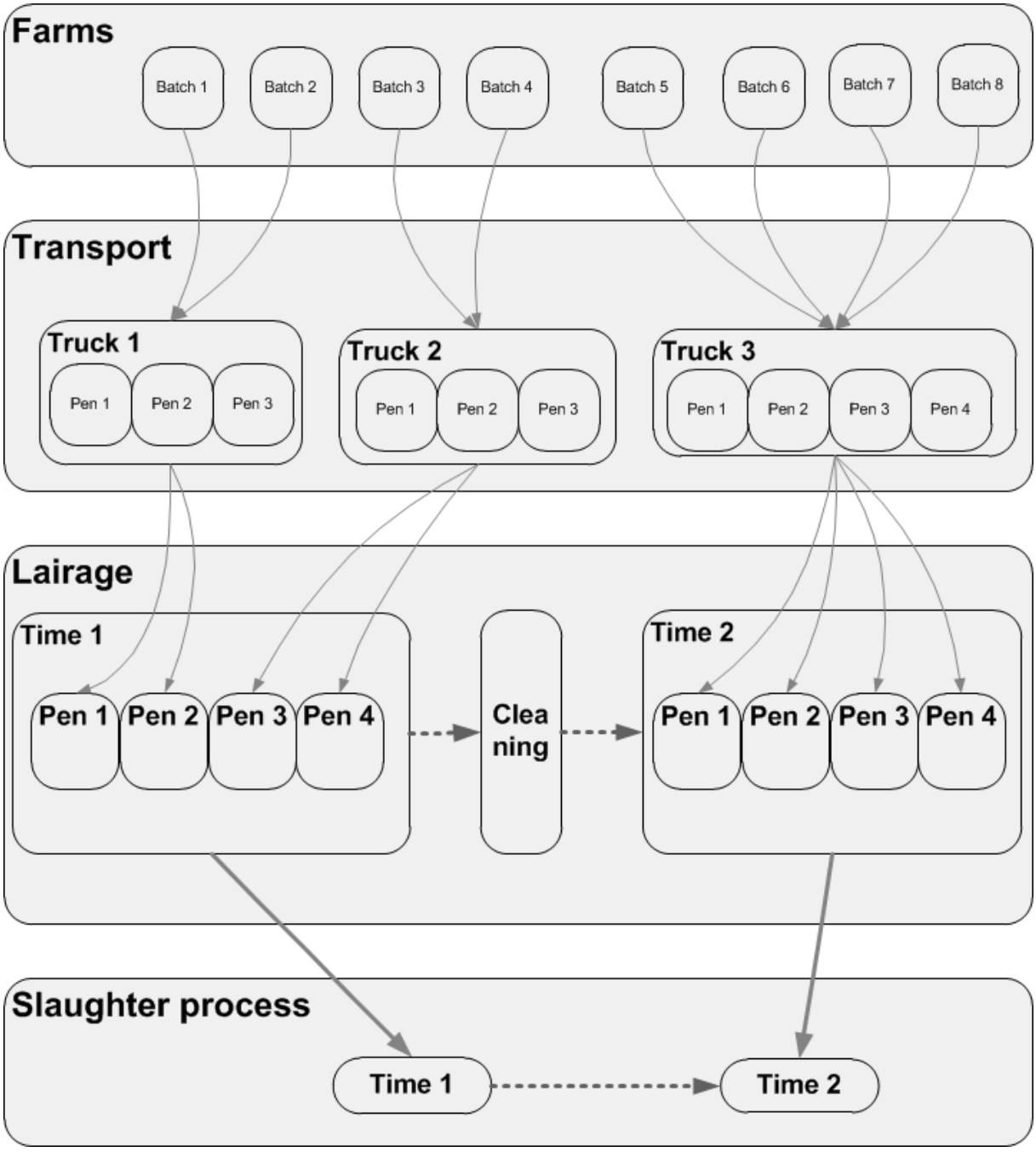

Figure 2: Theoretical example of Transport & Lairage model process at two time points during the day. Filled arrows indicate movement of pigs, dotted arrows passage of time.

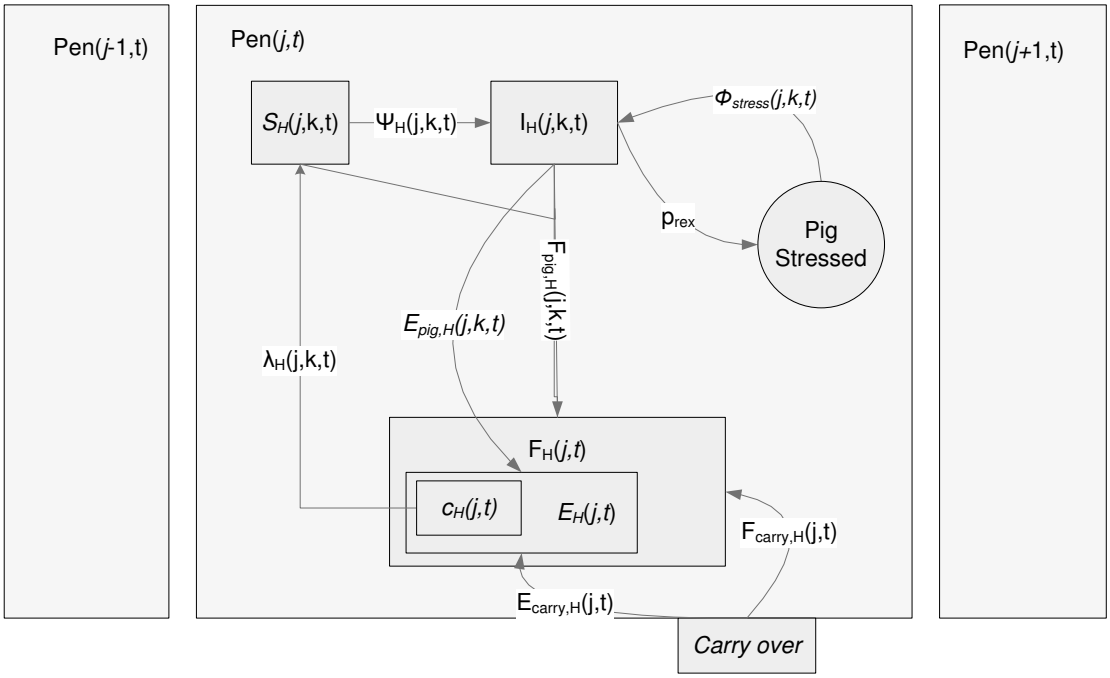

Figure 3: Schematic diagram of faeces (*F*) and *Salmonella* (*E*) transmission between Susceptible (*S*) and infected (*I*) pigs, in pen *j* at time *t* during stage *H* (Transport or Lairage), note stress only applies to the Transport stage. When pigs enter the pen there may already be some faeces (F_{carry}) and *Salmonella* (E_{carry}) present. While in the pen all pigs excrete faeces, F_{pig}, with faeces from infected pigs containing *Salmonella*, E_{pig}. Susceptible pigs may ingest a dose of *Salmonella*, λ, dependent on the concentration in the faeces in the pen, c_H and become infected with probability Ψ. Infected pigs may become stressed with probability p_{rex}, affecting the amount of *Salmonella* shed, Θ_{stress}.

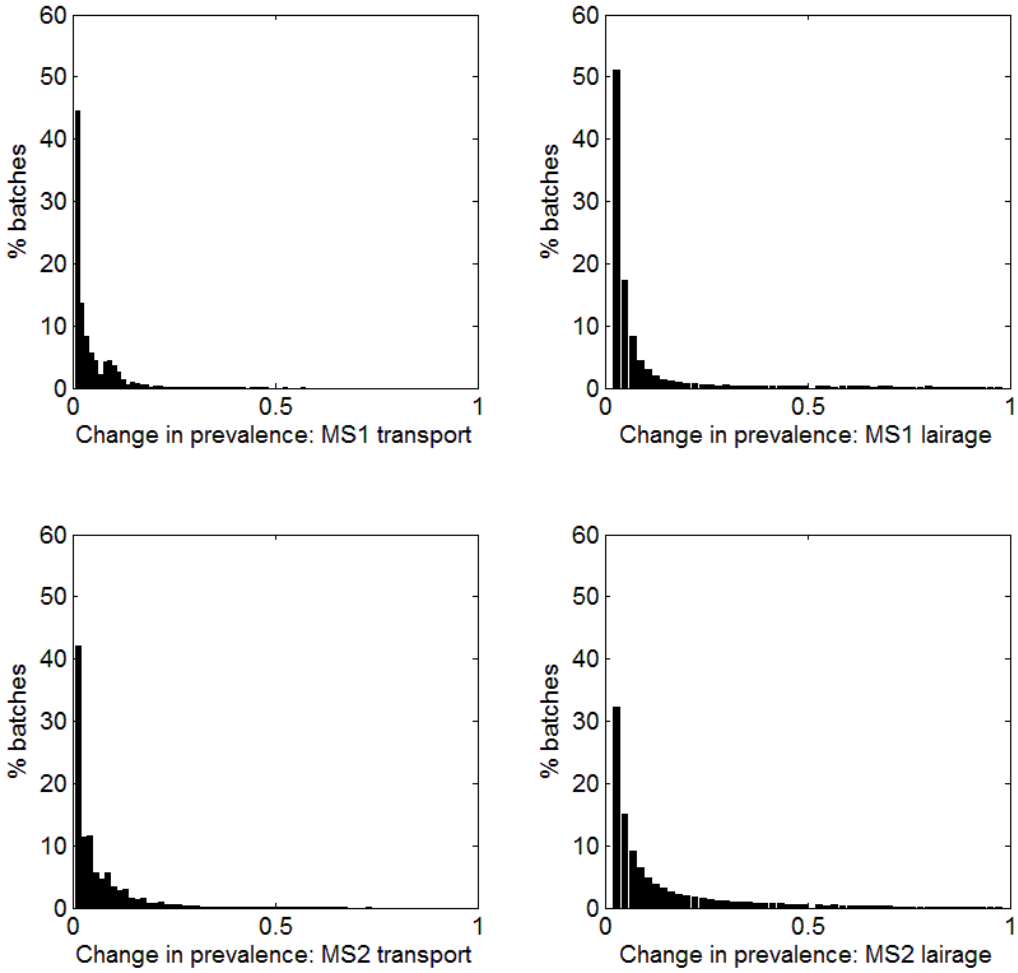

Figure 4: Distribution of nonzero changes in within-batch prevalence during transport (left) and lairage (right), for Member State 1 (MS1) (top) and Member State 2 (MS2) (bottom).

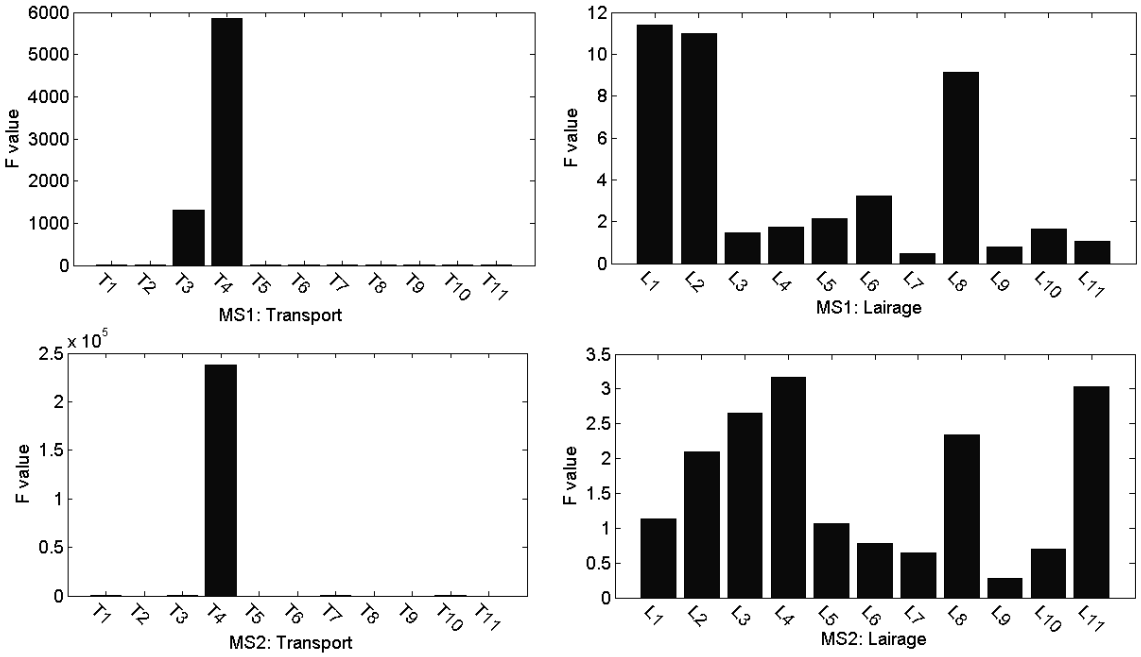

Figure 5: Transport & Lairage sensitivity analyses both Member States (MS1 and MS2). Descriptions of the variable labels are in Table IV and Table V.